ASSESSMENT OF ISLET ISOLATION EFFICACY IN DOGS

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VARIABILITY OF ISOLATION OUTCOME

- A major problem hampering human islet Tx

- Difficult to analyse due to the intertwined effects of numerous variables, like:
  - predonation events
  - donor & pancreas characteristics
    (islet content of pancreas)
  - organ preservation conditions
  - isolation methods

Knowledge of variability due to *intrinsic* factors like the islet content of the individual pancreas is essential for analysis of the extrinsic factors

PARAMETERS FOR ISOLATION EFFICACY

- **Insulin extraction**

  In the past, variability of pancreatic islet content was, indirectly, calculated in by estimating islet yield from insulin recovery from the pancreas

(insulin recovery may not accurately reflect islet yield)

- **Islet sizing ('morphometry')**

  - to assess final isolated islet yield
  - no attempts to compare islet yield with the islet volume of the individual pancreas

OUR STUDY

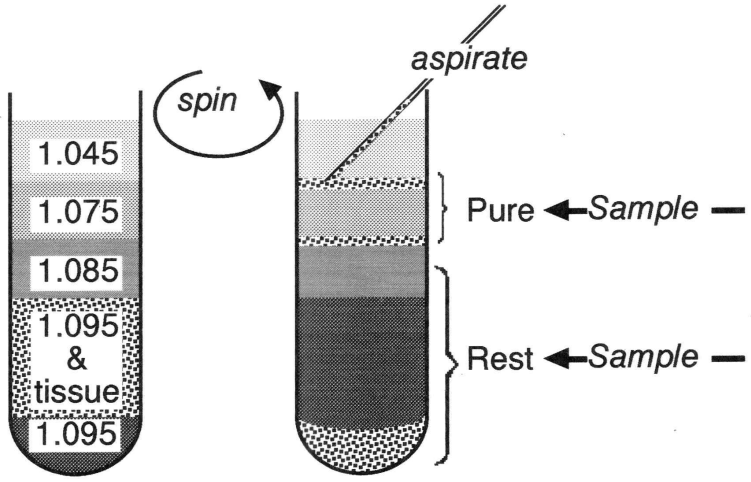
- We studied the impact of interindividual differences
  - pancreatic islet content
  - other donor characteristics (body wt, age)

  on isolation outcome
- compared morphometric and biochemical assessment
- new approach: introduction of the UW solution for islet preservation during the cold phase of isolation

Results from 31 consecutive islet isolations from the splenic segment of the dog panceas

ISLET ISOLATION METHOD

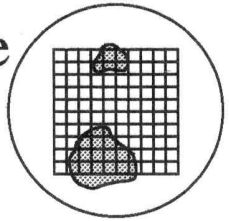
- Splenic pancreas (n=31) ← *sample Pancreas*
- Intraductal stationary digestion
  - collagenase-HBSS, 20 min at 38°C
  - decant
- Dispersion in cold UW solution
  - discard duct & vessels
  - syringe and screen 400 µm ← *sample Digest*
- Density gradient purification
(dextran-HBS, starch-UW or Percoll-UW)



For assessment samples were taken from the Pancreas, the Digest, and from the Pure and Rest fractions of gradients

ASSESSMENT

- Morphometry to assess islet volume and size
  - for sizing on H&E-stained sections of the pancreas we used a grid
  - during isolation the mean Ø of DTZ-stained islets (entrapped islets –est.<20%– were not counted)

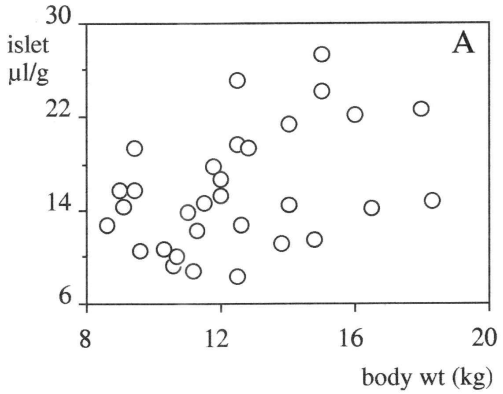


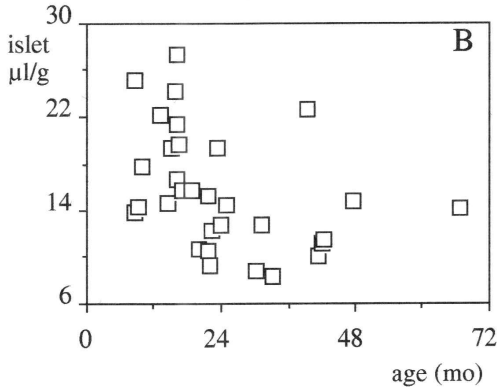
- Extraction for insulin and amylase recovery
- Microscopy of ß-cell granulation
- Islet function was studied by perifusion

Donor characteristics: age, body wt, and fractional volumes (Vv) of islet, fibrous and acinar tissue of the splenic pancreas in beagles (n =31)

	Mean	Range	**Variability**
Animal			
Weight (kg)	12.4	9 - 18	x **2**
Age (mo)	24.2	8 - 67	x **8**
Pancreas (Vv %)			
Islet	1.6	0.8 - 2.7	x **3**
Fibrous	8	5 - 12	
Acinar	90	87 - 92	

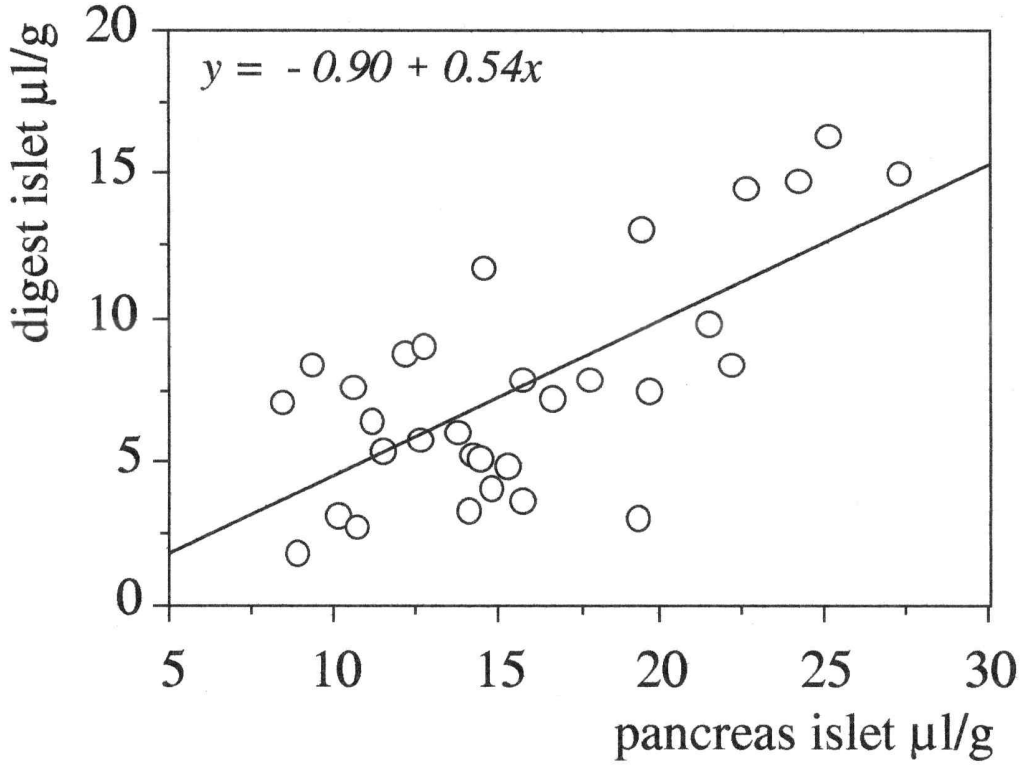
Correlations between body wt (A) or age (B) and the islet content of the splenic pancreas





Multiple correlation of the combined effects of body wt and age on islet content, r=0.75 (P<.0001)

Impact of islet content of the pancreas on outcome



Islet content of the pancreas vs. digest islet yield (r =.69, P<.0001).
Fractional islet volume explained 50% (r^2) of the variance in islet yield.

Recovery of total tissue volume, and amylase insulin and islet volume (per gram pancreas) in the digest, and pure and rest fractions of gradients

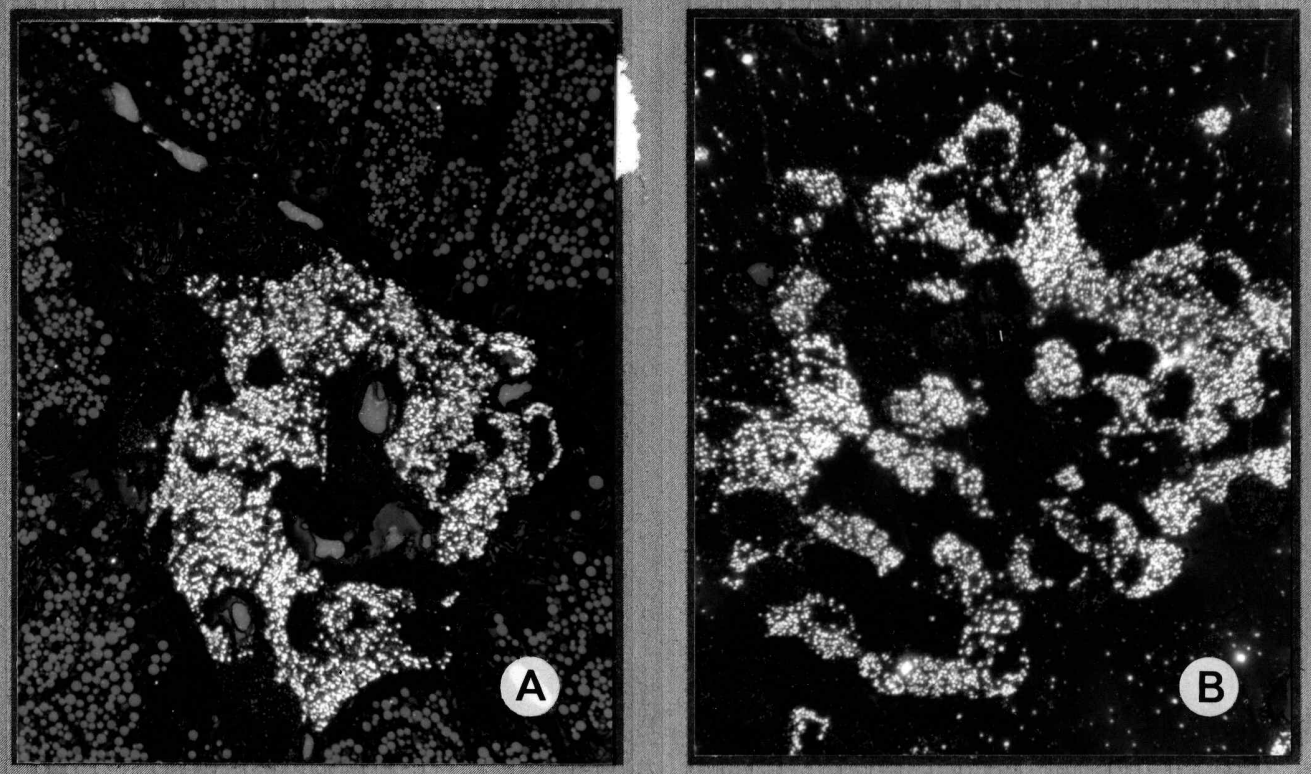
	Pancreas	Digest	Pure	Pure&Rest
Tissue (ml)	9.2	7.2*	<0.1*	5.2† (n=8)
%		**79**		**78**
Amylase (U)	7097	6706	1*	3939*
%		**95**	**.02**	**64**
Insulin (nmol)	21.6	18.7†	6.5*	15.3†
%		**89**	**36**	**83**
Islet (µl)	15.7	7.6*	3.9*	4.6*
%		**49**	**53**	**64**
Insulin/µl islet	1.5	2.9*	1.8*	ND
Purity (%)	1.57	ND	94	ND

% Recovery in the Digest compared to Pancreas values

% Recovery in the gradients compared to Digest values

*P<.001; †P<.05

Reflection contrast micrographs of sections of the pancreas (A) and purified islets (B) stained by the immuno-gold method for insulin



MORPHOMETRY *vs* INSULIN EXTRACTION

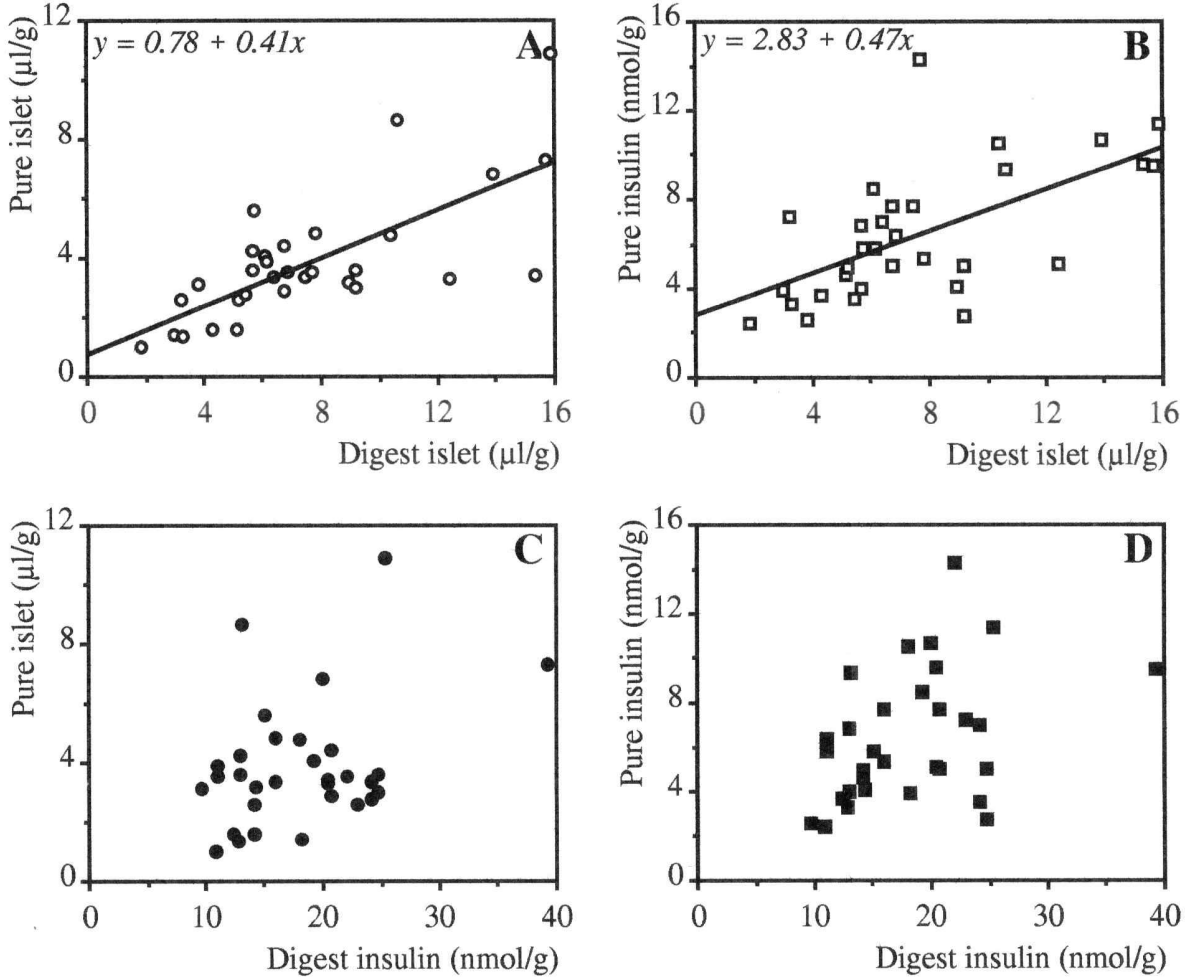
Correlation analysis (*r*) of:

1) Islet *vs* insulin measures in pancreas, digest or pure islet suspensions
2) Pancreas islet or insulin content *vs* yield during isolation
3) Digest islet and insulin *vs* values after purification

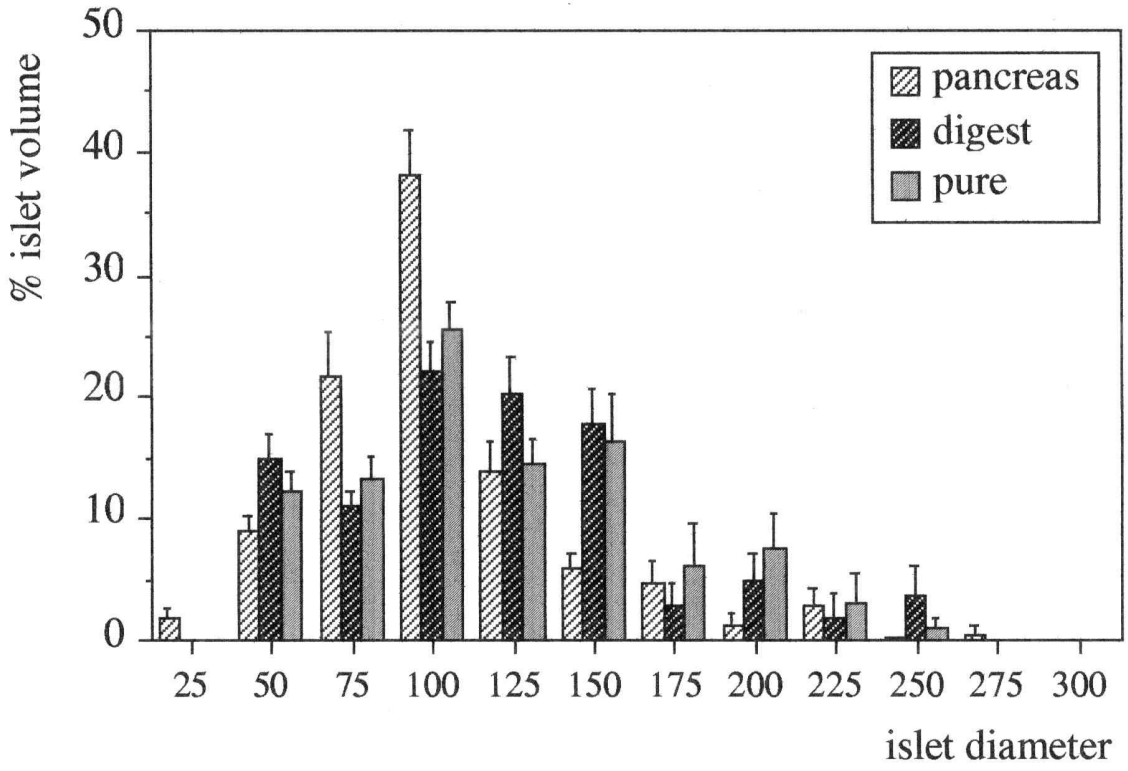
	Pancreas		Digest		Pure
	islet	insulin	islet	insulin	islet
Pancreas					
insulin	**.74***				
Digest					
islet	.69*	.75*			
insulin	.57*	.71*	**.63***		
Pure					
islet	.55*	.56*	.71*	.34	
insulin	.44†	.15	.65*	.40	**.59***
Pure & Rest					
islet	.53*	.48†	.58*	.37	
insulin	.16	.18	.23	.63*	

*$P<.001$; †$P<.01$

Comparison of islet sizing and insulin extraction for assessment

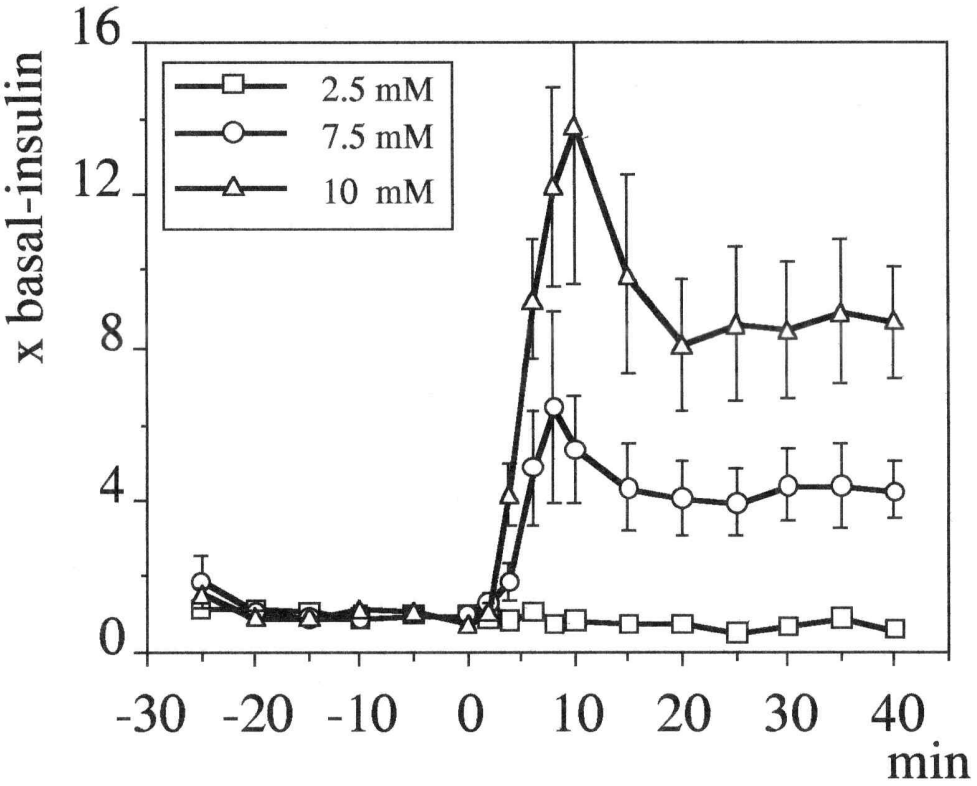


Volume size distributions of islets



A similar average diameter of islets in the pancreas and after isolation in the digest and purified suspension demonstrated no fragmentation

Insulin response to perifusion with glucose



Aliquots of the islet suspension were perifused in parallel, initially with 2.5 mM glucose, and from 0 to 40 min with 2.5 to 10 mM glucose (n=6)

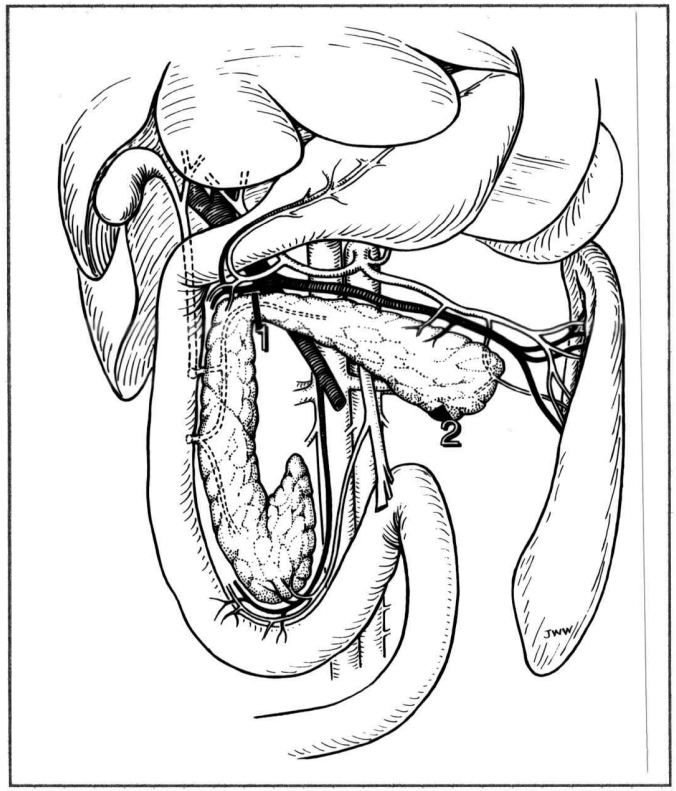
WE CONCLUDE

- The variability of islet and insulin yield, and size of isolated islets, may be attributed to a large extent to the variability of the native endocrine pancreas
- Isolation efficacy best documented by morphometry of the isolated and native islet population
- Additional insulin extraction allowed quantitation of entrapped islets—which we had underestimated (subjectively) during sizing—and further documented preservation of ß-cell granulation during isolation
- Similar studies should facilitate analysis of other factors affecting isolation outcome in man

Schematical representation of the beagle pancreas

- In control dogs the insulin distribution was studied from biopsies at sites 1 and 2
  - no significant difference was found

- In experimental dogs the pancreas was transected at site 1
  - a biopsy was taken from the cut end for assessment



Analysis of the effects of body wt and age on fractional islet volume of the pancreas and digest islet yield (n = 31) by multiple correlation (r) and regression (b')

Donor		Islet volume per gram pancreas	
		Pancreas	Digest
Body wt	(b'_w)	*.72**	.62*
Age	(b'_a)	*-.71**	-.67*
Body wt & Age	(r)	.75*	.68*
	(r^2)	**.57**	**.46**

Relative importance of body wt and age is indicated by beta-weights (b') of the regression equation: $I = b'_w \cdot W + b'_a \cdot A$ with standardized units for islet-volume (I), body wt (W) and age (A).

$\Rightarrow I = \mathit{0.72} \cdot W - \mathit{0.71} \cdot A$

Together body wt and age explained ~**50%** (r^2) of variability. *P<.001